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14. ABSTRACT
Hemorrhage is a leading cause of deaths on the battlefield. An understanding of the mechanisms and modulators of coagulopathy under conditions soldiers currently experience on the battlefield is important for improved treatment of the hemorrhaging soldier. The global objective of this project tests the hypothesis that environmental and physiological conditions a soldier experiences on the battlefield alters hemodynamic and hemostatic function (i.e., coagulation and fibrinolysis). During the current funding period we completed the objectives in specific aim 1A (test the hypothesis that passive heat stress alters hemostatic function during simulated hemorrhage) and are well on our way towards completing the objectives of aim 1B (Dehydration during exercise in the heat alters hemostatic function during simulated hemorrhage). For Aim 1A, subjects completed four visits (familiarization, a passive heat stress, a normothermic control, and a time control). For Aim 1B, each subject will likewise complete four visits (familiarization, exercise without dehydration, and two exercise with dehydration trials). TEG based assays have been obtained while plasma-based assays for Aim 1A are currently being run in at USAISR. We anticipate the plasma based assays for Aim 1B will be run by USAISR in the Fall of 2014 through early Winter of 2015.

15. SUBJECT TERMS
hemorrhage, hyperthermia, heat stress, coagulopathy, hemostasis.

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Introduction

Worldwide, trauma is the cause of 1 in 10 deaths, with 30-40% of trauma deaths being due to hemorrhage. Hemorrhage is also a leading cause of death on the battlefield. An understanding of the mechanisms and modulators of coagulopathy under conditions soldiers often experience on the battlefield is important to improve medical treatment of the hemorrhaging soldier. The global objective of this project is to test the hypothesis that environmental and physiological conditions a soldier experiences on the battlefield alters hemodynamic and hemostatic function (i.e., coagulation and fibrinolysis), resulting in compromised ability to survive a hemorrhagic injury. This objective will be accomplished by evaluating the following Specific Aims: 1A) Passive heat stress alters hemostatic function during simulated hemorrhage. 1B) Dehydration during exercise-induced hyperthermia alters hemostatic function during a subsequent simulated hemorrhage. 2) Heating a hemorrhaging individual who is not hypothermic is detrimental to blood pressure control, cerebral perfusion, and hemostatic function. A secondary objective of this work is to evaluate the effectiveness of two pre-hospital devices that are designed to provide the caregiver information regarding the hemorrhagic status of an individual. This project will provide the Department of Defense with valuable information resulting in improved medical treatment of soldiers who have experienced a hemorrhagic injury while in hyperthermic environmental conditions.

Body

The global objective of this project tests the hypothesis that environmental and physiological conditions, often experiences on the battlefield, alters hemodynamic and hemostatic function. The following are the items under the Statement of Work that we proposed would be accomplished during Year 2 of the project:

1) Submit and present work from specific aim 1A to a national meeting.
2) Accomplish the objectives outlined in specific aim 1B.
3) Analyze and interpret data obtained during the experiments outlined in specific aim 1A.
4) Write technical reports and/or scientific publications to disseminate information obtained from Specific Aims 1A and possibly 1B.

1) Submit and present work from specific aim 1A to a national meeting.

The following presentations originating from the funded project have either been presented in 2013/2014 or will be presented this spring (2014) at national meetings.

"Hyperthermia does not alter TEG-based markers of hemostatic function during simulated hemorrhage in humans." Craig G. Crandall, Zachary J. Schlader, Eric Rivas, Andre P. Cap, Victor A. Convertino.

"Heart rate response during hyperthermic lower body negative pressure: Implications for tolerance." Zachary J. Schlader and Craig G. Crandall.

"Does hyperthermia alter TEG-based markers of hemostasis during simulated hemorrhage in humans?" Daniel Gagnon, Zachary J. Schlader, Eric Rivas, Jena Langlois, Naomi Kennedy, Andrew P. Cap, Victor A. Convertino, Craig G. Crandall.

"Muscle oxygen saturation during hyperthermic central hypovolemia." Zachary J. Schlader, Eric Rivas, Naomi Kennedy, Jena D. Kern, Babs R. Soller, Victor A. Convertino, Craig G. Crandall.

"Dehydration associated with a simulated foot patrol in the heat impairs tolerance to a hemorrhagic insult." Craig G. Crandall, Zachary J. Schlader, Eric Rivas, Daniel Gagnon, Victor A. Convertino. (acceptance for presentation pending)

2) Accomplish the objectives outlined in specific aim 1B.
These studies will investigate the effects of exercise, with and without dehydration, on markers of hemostatic function during simulated hemorrhage.

Below is a graphical depiction of the protocol for aim 1B.

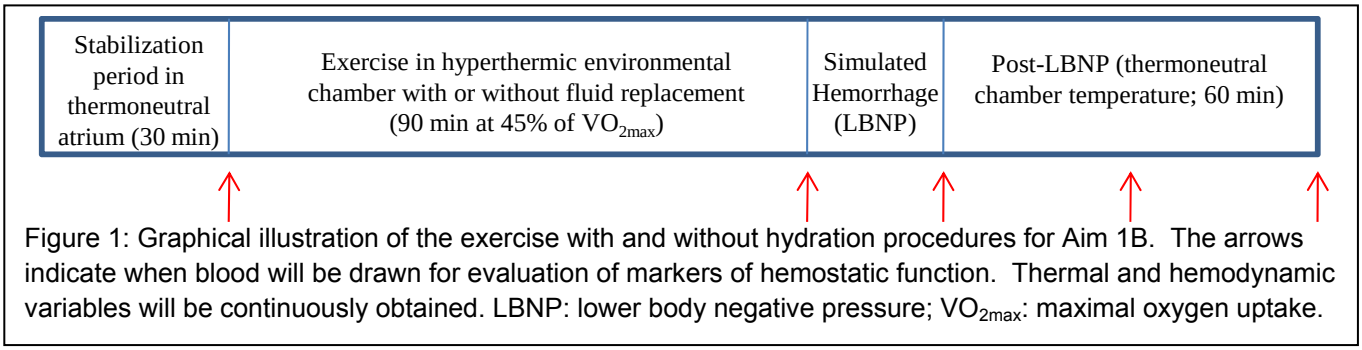


Figure 1: Graphical illustration of the exercise with and without hydration procedures for Aim 1B. The arrows indicate when blood will be drawn for evaluation of markers of hemostatic function. Thermal and hemodynamic variables will be continuously obtained. LBNP: lower body negative pressure; VO_{2max}: maximal oxygen uptake.

Subjects exercised for 90 min in the heat on three occasions: 1) fluid intake was sufficient to offset sweat losses, 2) fluid was withheld throughout, with the subject exercising until they achieved the same increase in internal temperature as #1 above, and 3) fluid was withheld throughout and the subject exercised for the full 90 min. Please note that the third limb above was an addition to the original protocol given observations originating from limbs 1 and 2. The addition of this limb extended the duration required to complete this aim, and thus this aim is not yet completed. Due to the need to wait ~60 days between trials for each subject, we project this aim will be completed in early to mid-Fall 2014. Given that this aim is not completed, we do not have complete data regarding the effects of the applied perturbations on markers of hemostasis. However, below is an abstract submitted to the Military Health System Research Symposium (2014) that addressed the impact of fluid on simulated hemorrhage tolerance between limbs 1 and 2.

Purpose: This project tested the hypothesis that dehydration associated with moderate exercise in hot environmental conditions impairs the capability to tolerate central hypovolemia, as would occur during a hemorrhagic insult.
Methods: On two occasions 10 male subjects (34±7 years) exercised on a treadmill in an environmental chamber set at 40 °C (104 °F) and 30% relative humidity. For the first trial, subjects exercised for 90 min while ingesting fluid to offset sweat loss (Hydrated Trial). For the second trial, separated by ~2 months, fluids were withheld throughout the protocol (Dehydrated Trial), while the subjects exercised to the same increase in internal temperature as the first trial.

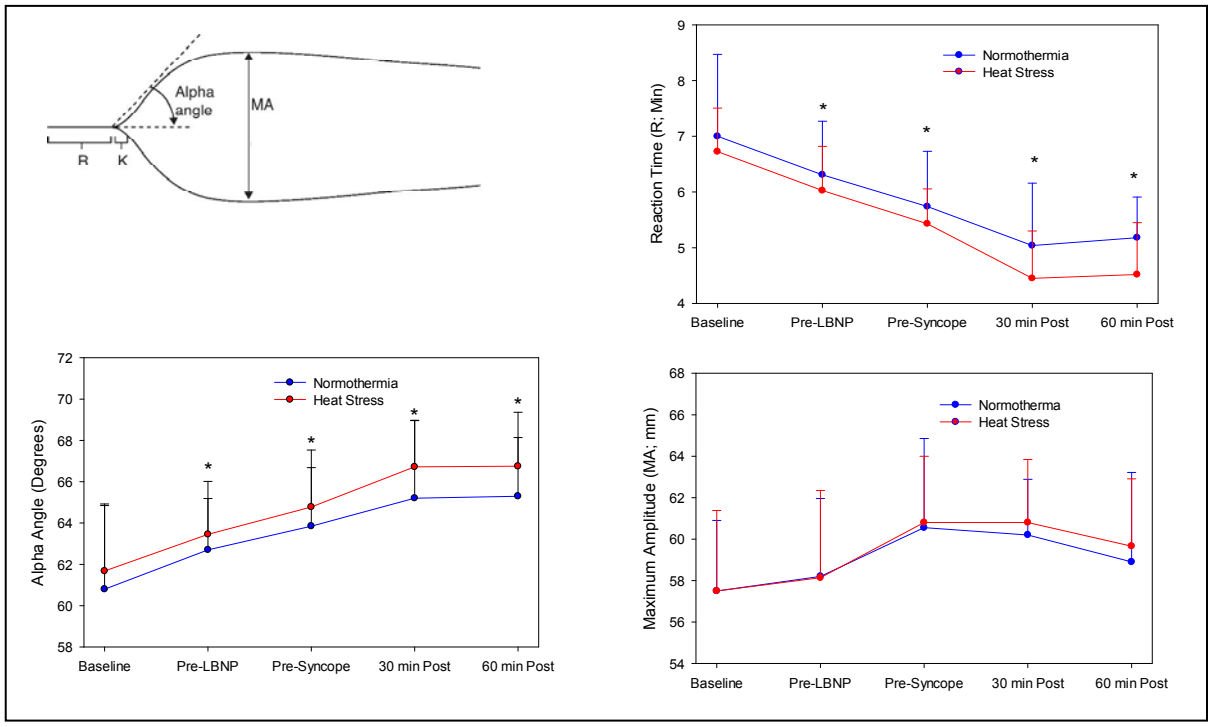
The imposed workload was comparable to a routine foot patrol and was identical between trials (oxygen consumption: 1.74±0.37 L/min; P=0.8 between trials). Immediately upon cessation of each exercise trial, subjects were exposed to a simulated hemorrhagic challenge via progressive lower-body negative pressure (LBNP) to pre-syncope. LBNP tolerance was quantified via a cumulative stress index (CSI), which was calculated by summing the product of the LBNP level and the time at each level to pre-syncope (e.g., 3 min x 20 mmHg LBNP + 3 min x 30 mmHg LBNP, etc.). **Results**: By design, the increase in internal temperature at the end of exercise but prior to LBNP was identical between trials (both 1.2±0.4° C), while body mass was reduced by 0.7±0.3 kg in the Dehydrated Trial but was unchanged in the Hydrated Trial. LBNP tolerance was reduced by ~25% in the Dehydrated Trial (Dehydrated Trial CSI: 419±191 units, Hydrated Trial CSI: 589±217 unit; P=0.03). **Conclusion**: These data suggest that inadequate fluid intake resulting in relatively mild dehydration during a foot patrol in conditions of elevated environmental temperature may compromise the capacity to withstand central hypovolemia, as would occur during a hemorrhagic insult.

3) Analyze and interpret data obtained during the experiments outlined in specific aim 1A.

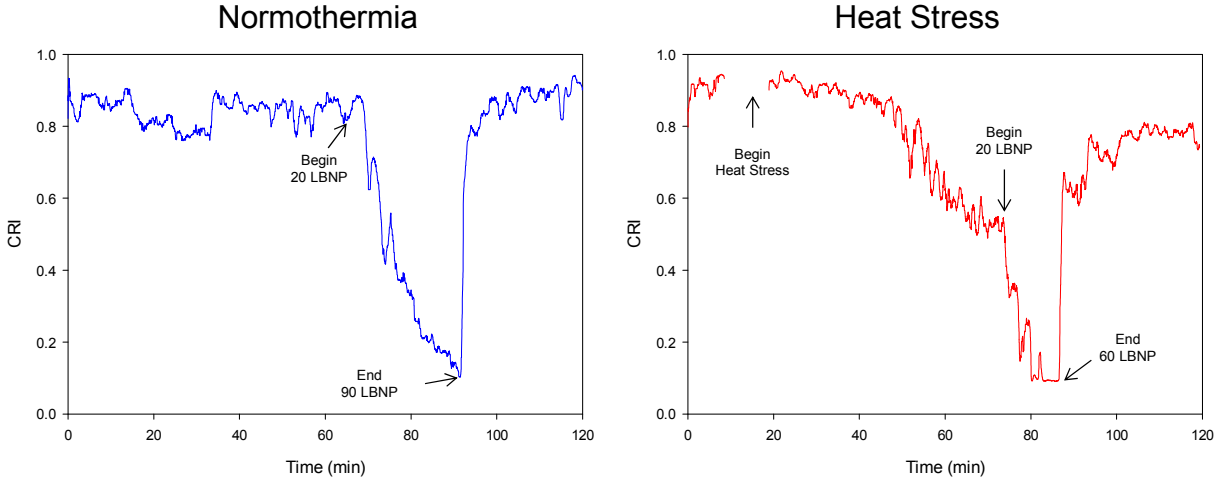
We have completed the analysis of the TEG data for this limb. Below is text from an abstract that will be presented at a meeting this spring pertaining to those data, as well as figures that depict those findings. The plasma based assays will be run at the USAISR. We anticipate completion of those assays by early-summer.

Abstract: Hyperthermia and central hypovolemia independently activate the coagulation system. However, their combined effects on hemostatic function remain relatively unknown. **PURPOSE:** We examined the hypothesis that hyperthermia, in combination with a simulated hemorrhagic challenge, alters markers of hemostasis. **METHODS**: On separate, randomized days, 12 healthy male subjects (32 ± 6 yrs) underwent either passive heat stress to increase intestinal temperature by 1.2°C (hyperthermia) or remained normothermic for a time control period. This was immediately followed by progressive lower body negative pressure (LBNP) until pre-syncope. Blood samples were obtained at the end of baseline rest, prior to LBNP, immediately post-LBNP, and 60 min post-LBNP. Blood samples were analyzed using thromboelastography (TEG) to determine coagulation time (R), clot formation time (K), the kinetics of clot development (α angle), and maximum clot strength (MA). **RESULTS**: For each variable, there was a significant effect of time (all $p \leq 0.001$), however these changes did not differ between thermal conditions (*time×condition* interactions all $p > 0.1$). As such, the data presented are collapsed across both thermal conditions. Overall, R and K decreased from 6.9 ± 1.2 min and 2.2 ± 0.3 min at baseline to 5.6 ± 0.8 min and 1.9 ± 0.2 min post-LBNP, respectively. In contrast, α angle and MA increased from 61 ± 4° and 57.5 ± 3.6 mm at baseline rest to 64 ± 3° and 60.7 ± 3.7 mm post-LBNP, respectively. R (4.8 ± 0.9 mm), K (1.7 ± 0.2 mm), α angle (66 ± 3°), and MA (59.3 ± 3.7 mm) remained significantly different from baseline at 60 min post-LBNP (all $p < 0.05$). **CONCLUSIONS**: These data show that hyperthermia in combination with a simulated hemorrhagic challenge

does not uniquely alter TEG-based markers of hemostasis relative to a comparable challenge when individuals are normothermic.



We have also evaluated the effects of heat stress on CipherOx derived compensatory reserve index (CRI) during lower-body negative pressure to pre-syncope. Below are figures from a subject that generally represents the findings, although data for some subjects were more variable. These data suggest that heat stress itself reduced the CRI well prior to the simulated hemorrhagic challenge.



CipherOx data during heat stress and time control (normothermia) followed by LBNP

4) Write technical reports and/or scientific publications to disseminate information obtained from Specific Aims 1A and possibly 1B.

We are unable to write up the primary data from Aim 1A given that we are waiting on the plasma samples to be assayed at the USAISR. The residual of the data have been reduced. As soon as we get the plasma assays samples from the Army, we will be prepared to write up and submit this manuscript. That said, we have written a manuscript revolving around the capacity of the Reflectance Medical device to predict hemorrhagic state during lower-body negative pressure (LBNP) while heat stressed. We anticipate submitting that work for publication late spring 2014. We had one paper published (see citation below) that included data from Aim 1A, although the objective of that work was peripheral to the project funded by the Army.

Normothermic central hypovolemia tolerance reflects hyperthermic tolerance. Schlader, Z.J., C.G. Crandall. *Clin Auton Res* 2014 (in press).

Due to the requirement to add an additional limb to Aim 1B, we don't anticipate that we will have the complete dataset to work on publications pertaining to that aim until late 2014 to early 2015.

Planned work during the ensuing 12 months:
We will complete data collection for the current protocol (aim 1B) in fall 2014. However, like data from aim 1A, we are reliant upon laboratory personnel at the USAISR to run the plasma-based samples for that protocol. We will work closely with that laboratory to see that those samples are run in a timely manner. We will work with Flashback Technologies to assist in the analysis of the CRI data from both protocols 1A and 1B. We will initiate data collection for Aim 2, which will investigate whether warming a person undergoing a hemorrhagic insult is detrimental to the control of blood pressure and cerebral perfusion. Finally, we anticipate the submission of at least 2 manuscripts from data originating from protocols 1A and 1B during the ensuring 12 months.

Key Research Accomplishments

- Completed data collection for Aim 1A.
- Close to completing data collection for Aim 1B.
- Published a manuscript from data originating, in part, from the present funding.
- Presented findings originating from this work at national meetings.
- Submitted findings for consideration for presentation at national meetings in 2014.

Reportable Outcomes

- The obtained data were submitted for presentation at the 2014 Military Health System Research Symposium.
- Upon completion of data collection and analysis, multiple manuscripts will be written covering the findings of this work.
- Eric Rivas, MS, is a minority doctoral student at Texas Woman's University who will conduct his doctoral dissertation studies in Dr. Crandall's laboratory. Mr. Rivas is funded in part through grant dollars originating from this project.

Importantly, this project is giving Mr. Rivas valuable laboratory experience in preparation for his doctoral studies.

Conclusions

The obtained data indicate that passive heat stress (as would be experienced by a gunner on a vehicle, a sniper, or any other condition where a soldier is exposed to the sun and thus is passively heated) does not alter whole-blood based markers of hemostatic function alone or following a simulated hemorrhagic challenge. Assuming these findings will be consistent with plasma-based markers of hemostasis, these data will benefit those who treat the hemorrhaging soldier in the field by informing them that no modification in hemostasis control is needed when an injured soldier is heat stressed. Perhaps just as valuable as these data will be the evaluation of the results from the Flashback CipherOx device in predicting the extent of a hemorrhagic injury in the heat stressed soldier. Should it be found that heat stress modifies the algorithms used to predict hemorrhagic status by this device, this would be critical information to implement into the devices before they are used in combat situations under conditions of elevated environmental temperatures. Finally, data from Aim 1B strongly suggests that dehydration associated with moderate exercise (as would occur during foot patrols), even when accounting for the magnitude of the increase in internal temperature, will compromise the ability of a soldier to tolerate central hypovolemia as would occur during a hemorrhagic injury.

References

Schlader, Z.J., Crandall, C.G., Normothermic central hypovolemia tolerance reflects hyperthermic tolerance. Clin Auton Res 2014 (in press).

Crandall, C.G., Z.J. Schlader, E. Rivas, A.P. Cap, V.A. Convertino. Hyperthermia does not alter TEG-based markers of hemostatic function during simulated hemorrhage in humans. Military Health System Research Symposium. 2013.

Schlader, Z.J., C.G. Crandall. Heart rate response during hyperthermic lower body negative pressure: implications for tolerance. *Clin Auton Res*: 23: Page 265, 2013.

Crandall, C.G., Schlader, Z.J., Rivas, E., Gagnon, D., Convertino, V.A. Dehydration associated with a simulated foot patrol in the heat impairs tolerance to a hemorrhagic insult. Submitted to Military Health System Research Symposium. 2014.

Schlader, Z.J., Rivas, E., Kennedy, N., Kern, J.D., Soller, B.R., Convertino, V.A., Crandall, C.G. Muscle oxygen saturation during hyperthermic central hypovolemia. *The Faseb J* (in press).

Gagnon, D., Schlader, Z.J., Rivas, E., Kern, J.D., Kennedy, N., Cap, A.P., Convertino, V.A., Crandall, C.G. Does hyperthermia alter TEG-based markers of hemostasis during simulated hemorrhage in humans? *Med. Sci. Sports Exerc (in press)*

Appendices
None

Supporting data
See data in the body of the text above.